NON-FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH

Panel Manager - Dr. Bernard Y. Tao, Purdue University
Program Director - Mr. Jeffery L. Conrad

Agricultural commodities can provide the raw materials for production of numerous industrial and consumer products such as lubricants, fuels, paints, detergents, biodegradable polymers, textile fibers, fiber composites, pharmaceuticals, and various other commodity and specialty chemicals. The Non-Food Characterization/ Process/Product Research program supports research on improved methods for producing existing non-food, agriculturally-derived products and on developing new, non-food uses for agricultural commodities. Research seeks to better understand properties of agricultural materials related to their quality, value, and processing characteristics and to develop innovative products and processes for conversion of agricultural materials to non-food products.

This program also supports biofuels research directed toward understanding and overcoming factors which limit the technical and economic efficiency of production of alcohol fuels and biodiesel. Supported research focuses on the physiological, microbiological, biochemical, and genetic processes and mechanisms controlling the biological conversion of agriculturally important biomass material to alcohol fuels.

2000-01921 Silica Based Industrial Products from Rice Hull Ash Silica

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Grant 2001-35504-10044; $133,000; 2 Years

In 1999, 9.5 million metric tons of rice was produced in the United States. Twenty percent of the grain is low value rice hulls that have posed a significant waste disposal problem for the US rice industry for many years. To alleviate the disposal problem, many rice millers have developed controlled combustion technologies to burn hulls as a source of fuel and reduce the volume of waste. The rice hull ash produced comprises of about 19% hull and consists of 93-97% amorphous silica. The ash is used as a refractory material in the steel industry but there are limited uses for this product and the rice industry is seeking new markets. Amorphous rice hull silica is readily soluble in dilute alkali and could be used as novel raw material for ceramics production. The advantage of this technology, relative to current production methods, is the simplicity of extraction of amorphous silica that does not require a traditional smelting process, which is required when using sand as the conventional raw material. Silica gel and plastic sheets have been produced in laboratory studies from rice hull ash using this novel technology. The proposed research anticipates converting rice hull ash to ceramic materials by dissolving in alkaline solution, subsequent precipitation and drying to produce a lightweight material. The effect of silica solution concentration and conditions of precipitation on the structure and physical properties of the ceramic products will be studied. Industrial applications for the novel materials will then be identified.

2000-1901 Combinatorial Biological, Chemical, and Engineering Approaches for Agriculture and Food

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Conference Grant; Grant 00-35504-9346; $10,000; 1 Year

Much of the progress in growing and converting agricultural crops to food and non-food products has relied on traditional, rational approaches: i.e., beginning with what is known and taking reasoned steps towards new solutions. The new approaches are generally adaptations of existing approaches, and are within the intellectual comfort zone of the scientist and within the funding zone of the supporting organization. Within the last 10 years scientists have come to recognize that a large number of possibilities have been overlooked and that a vast unexplored idea space needs to be explored in order to meet the needs of science and society in the future. Fortunately, advances in miniaturization, robotics, and genetics have provided tools that can open windows on this idea space to reveal truly innovative and in some cases totally unexpected results. Combinatorial methods use these advances to generate a very large, or Saganesque number of possibilities that are evaluated at small or micro scales. The methods are applied in developing enzyme variants, pharmaceutically active compounds, polymer formulations, antimicrobial compounds, etc. and have led to enzymes active under conditions that were previously inhibitory, extremely effective pharmaceuticals, and unique polymers. The utility of these methods is newly recognized and the potential for, and early success with applications to agriculture, crop conversion, and foods will be described and explored in the subject symposium.

2000-01873 Chemical Modification of Biodiesel by Oxidative Cleavage

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Strenthening Award; Grant 2001-35504-10177; $144,132; 2 Years

Biodiesel made from vegetable oils such as soybean oil has been proposed as an agricultural based substitute for petroleum diesel. Because of the high cost of agricultural oils, biodiesel is not cost competitive, and may not represent a new market for farmers. Biodiesel has shown reductions in particulate emissions relative to petroleum diesel, and is a renewable fuel having global warming and energy security benefits. The potential to produce biodiesel may equal 10% of the transportation diesel consumption in the U.S. The technical barriers to widespread use of biodiesel are: (1) high cost, (2) increased emissions of nitrogen oxides relative to petroleum diesel, (3) poor cold flow properties and (4) oxidative instability. We have developed a strategy for overcoming these problems by oxidative cleavage of the carbon-carbon double bonds in the fatty acid chains. The products of this process include biodiesel with improved emissions, cold flow properties, and oxidative stability, as well as several high value co-products. A two-step approach has been developed based on preliminary results. In step 1 catalytic oxidation of double bonds to produce epoxides, under acidic conditions where the epoxides are rapidly converted to diols, is performed. In step 2 the diols are catalytically cleaved with oxygen or air over a complex oxide catalyst. Development of reaction conditions and catalysts that maximize yields is the objective of this project.

2000-01922 Targeting Cellulases to Cellular Compartments in Plants for Bi ofuels Production

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Grant 00-35504-9369; $200,000; 3 Year

The long-term goal of this project is to develop an economical means of converting plant biomass to clean-burning renewable fuels, such as ethanol. We propose to generate transgenic plants that will produce thermostable microbial cellulases for this process. The enzymes will be inactive during growth of the plants but can be activated after harvest as a crude enzyme preparation suitable for industrial application. By reducing the process costs, this strategy will make biofuels more economical, thus creating a demand for bioenergy plants such as switchgrass and alfalfa that can thrive on marginal farmland and providing new uses for waste agricultural products. At the same time, promoting alternative fuels from renewable resources addresses problems associated with petroleum-based fuels: air and water pollution, accumulation of greenhouse gases, and U.S. dependence on an uncertain source of energy.

Our specific goals focus on targeting three thermostable bacterial cellulases to subcellular compartments in plant cells. We are developing versatile expression cassettes to direct these enzymes to the endoplasmic reticulum (ER), apoplast, neutral vacuole, and acidic vacuole. To test the feasibility of our targeting strategies, we will generate transgenic tobacco BY-2 cells for each enzyme/targeting strategy. We will determine subcellular localization of the recombinant proteins and compare recoverable enzymatic activity in subcellular compartments. We will determine the feasibility of expressing more than one cellulase in the same plant cel1 line, in the same or different compartments. Using El-cat as a prototype, we will also determine the best targeting strategies for this enzyme in *Arabidopsis thaliana* plants.

2000-01900 Improved Xylose Utilization for Ethanol Fuel Production by Ethanologenic *Escherichia coli*

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Postdoctoral Fellowship; Grant 2001-35504-10039; $90,000; 2 years

Enormous amounts of automotive fuel are imported and consumed annually. The imminent depletion of petroleum reserves, along with our economic dependence on foreign imports and the negative impact of automotive fuel combustion on the environment, demand that new technologies be investigated to provide alternative automotive fuels for future energy needs. The development of technologies to produce fuel ethanol from plant carbohydrates through microbial conversion processes could provide a clean renewable source of automotive fuel and concomitantly strengthen rural economies. This technology involves converting the fermentable carbohydrate components of plant biomass into ethanol using fermentative bacterial or fungal "biocatalysts". A unique property of one such biocatalyst, *Escherichia coli*, is its "metabolic flexibility": it can produce ethanol from most of the sugars commonly found in plant biomass. Ethanol conversion rates differ for different sugars, however. Pentoses, such as xylose, make up a substantial portion of fermentable plant carbohydrate, but are converted less rapidly than hexoses; these lower rates of conversion result in longer fermentation times and increased costs. The proposed research involves the metabolic engineering of ethanologenic *E. coli* to identify routes to increase the rate of xylose metabolism. Modifications in the expression/biosynthesis of native xylose uptake and metabolism genes/enzymes of *E. coli* will be made to allow higher rates of ethanol production from xylose. The use of such improved ethanologenic strains for ethanol production reduce costs during industrial scale fermentations. This study should also advance our basic understanding of carbohydrate metabolism for the production of other biobased products.

2000-01259 Micro-Crystalline Cellulose from Chemical-Free Pulping of Sugar-Cane Bagasse

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Seed Grant; Grant 2001-35501-10197; $62,830; 2 Years

The proposed research focuses on enhancing the value of an agricultural residue. Specifically, a novel pulping process that is inherently simple: brief exposure of the feedstock to hot liquid water (HLW) at elevated temperature, will be applied to sugar-cane bagasse. This process solubilizes much of the bagasse (up to 50 %), leaving a fibrous residue rich in cellulose. The second step in the process is chlorine-free bleaching, to remove the remaining non-cellulosic material. Lastly, the cellulose is purified and transformed to produce micro-crystalline cellulose (MCC). MCC is currently produced from wood by chemical pulping processes and used in the pharmaceutical and nutraceutical industry as a binding agent. The overall goal of this project is to establish the technical and economic feasibility of MCC production from an agricultural residue such as sugar-cane bagasse, via HLW pulping and chlorine-free bleaching. The four-task plan of work proposed to achieve this goal consists of: (1) an examination of the range of temperature/time/water-biomass ratios (consistency) possible with HLW pulping while still achieving the high solubilization of non-cellulosic components needed for production of MCC; (2) an examination of the additional purification possible by chlorine-free bleaching of sugar-cane bagasse HLW pulp; (3) the production and characterization of MCC from HLW pulping and chlorine-free bleaching of sugar-cane bagasse; (4) the completion of a pre-commercial market assessment

2000-01822 Development of a Transgenic Crop for the Production of Cationic Antimicrobial Peptides

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Grant 00-35504-9389; $102,000; 2 Years

Diseases such as AIDS, cancer and drug-resistant bacterial infections present a major threat to American health and agricultural industry. Third world diseases such as malaria and Chagas' disease continue to debilitate the health of the people living in these countries. The need is paramount for new drugs to treat disease and to replace and supplement conventional antibiotics, to which many disease-causing organisms have acquired resistance in the clinical and agricultural industries. A new family of drugs called cationic antimicrobial peptides show promise in combating these and other diseases. These drugs are found in natural secretions of most living organisms but are produced in extremely small quantities. Because they are expensive to make by chemical means their practical application to human and animal health will remain untapped until a method of mass production is developed. We propose to investigate the mass production of an important cationic antimicrobial peptide in the seeds of genetically engineered corn plants. Transgenic corn has been used for the commercial production of a number of biologically important molecules, and transgenic products produced in the seed of corn can be stored, distributed and extracted with relative ease. The U.S. capacity for corn production and the pressing need to add value to the crop makes engineering transgenic corn both practical and economical. This work will benefit U.S. agriculture by adding value to corn and the U.S. health industry by providing a source of a valuable new drug.

2000-01820 Enhancing Value of Tomato for Delivery of an Edible Vaccine

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Grant 00-35504-9347; $186,000; 3 Years

The overall goal of this project is to develop specialty tomato cultivars that produce fruits carrying a subunit vaccine to protect the human population against the respiratory syncytial virus (RSV) that causes pneumonia and bronchiolitis. This human pathogen is especially serious on infants, young children, and the very elderly. Of those infected with this virus, a mortality rate of 0.1-2.5% has been reported, and almost 100,000 cases of hospitalization have been reported in the United States alone. These figures are much higher in other parts of the world, especially in third-world countries. Earlier studies in our laboratory has demonstrated that transgenic tomato plants carrying an antigenic protein gene can produce this protein in the fruit, and when fruit is fed to mice, they induce an immune response. This proposed study is designed to increase the level of antigenic protein levels in tomato fruit, assess level of gene expression during fruit development and following storage, and conduct efficacy studies of this RSV edible vaccine. This tomato-based edible vaccine will increase the overall value of the tomato as it provides a new commercial use for this crop. This specialty tomato will be grown for the sole medicinal purpose of production and delivery of an edible oral vaccine against the serious human pathogen RSV, thus protecting the human population against this disease.

2000-01898 Simplified Technology for Enzyme Production with Thermophilic Anaerobic Bacteria

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Grant 2001-35504-10106; $195,000; 3 Years

Agricultural and forestry biomass are renewable resources for the sustainable production of fuels and other biochemicals. However, technical issues currently limit the economic feasibility of such bioconversion processes. The National Renewable Energy Laboratory Ethanol Project has identified cellulase (the main enzyme used in the pretreatment and degradation of biomass for biofuels) production as a high priority for research and development. Our proposal links (1) a new, non-food use for under-utilized biomass with (2) a novel method for supplying enzymes for biofuel and biochemical production. This process will likely result in improved methods for producing such chemicals and would lessen US dependence on foreign energy supplies. The results are also transferable to other processing industries that use enzymes, such as the beverage alcohols, animal feeds, paper processing, and detergent additives. The overall project goal is to evaluate the feasibility of using solid-state cultivation (SSC) of anaerobic, thermophilic bacteria as a production method for producing thermostable cellulase from agricultural residues. The objectives of the project are to: (1) develop a process for the production of bacterial biomass production using anaerobic solid-state cultivation techniques at high temperatures; (2) characterize the cellulase activity and thermostability; and (3) evaluate the economics of producing enzymes by SSC versus traditional methods. Several anaerobic, thermophilic bacteria will be screened for growth and cellulase production on agricultural residues. The economics of producing enzymes using SSC will be analyzed and compared to the current method of enzyme production, specifically for the biofuels application.

2000-01904 Condensed Phase Catalytic Hydrogenation of Crop-Derived Organic Acids

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Grant 98-35504-6356; $180,000; 2 Years

Organic acids are produced via fermentation of agriculturally-derived carbohydrates such as glucose, and constitute an important class of feedstocks for a renewable resource-based chemicals industry. Of the various reactions that these organic acids can undergo, hydrogenation is attractive as a potentially viable route to value-added chemicals that have traditionally been produced from petroleum. The major objective of this project is to establish the relationship between organic acid molecular structure and efficacy for hydrogenation at mild conditions, thus establishing a rational basis for selecting candidate acid feedstocks and for developing initial process design concepts. Organic acids to be investigated include amino acids, hydroxyalkanoic acids, and dicarboxylic acids. In addition to the monomeric form of these acids, oligomers of several acids will be investigated at conditions favoring simultaneous hydrolysis (breakdown to monomer) and hydrogenation to examine the idea of converting waste organic acid polymers into chemical products. In parallel to conversion studies, the reaction mechanism will be examined to explain how the molecular structure of the acid affects its reactivity. This project will fill a void in the fundamental knowledge base required for development of chemical processes for agriculturally-based feedstocks, thus enhancing the prospects for a renewable resource-based chemicals industry based on corn and other agricultural materials.

2000-01889 Use of Vegetable Oils in Metalworking Fluid Formulations

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Grant 2001-35504-10104; $120,000; 2 Years

More than 100 million gallons of concentrated metalworking fluids are sold annually to provide lubrication and cooling during machining of metal parts. The fluids used most commonly are mixtures of petroleum-based oil in water. These mixtures have two important drawbacks. First, machining generates a fine mist from the oil. Studies indicate that millions of workers exposed to these metalworking fluid mists may have elevated levels of respiratory ailments, dermatitis, and cancer. Second, these mixtures cost about $5 per gallon to dispose of properly because the fluids do not biodegrade readily.

Vegetable oils have the potential to replace petroleum-based oils in metalworking fluids. Because these oils are derived from renewable, non-toxic agricultural resources that biodegrade easily, the costs of disposing of these fluids would be lower than for existing fluids. In addition, the properties of vegetable oils suggest that fewer droplets would be formed during their use than with present fluids.

The goal of the proposed research is to formulate vegetable oil-in-water mixtures that can replace metalworking fluids used currently. Specific objectives include developing formulations of vegetable oil-in-water mixtures, screening the formulations by measuring fluid properties related to success during machining, and comparing droplet generation from the most promising formulations to current products. At the conclusion of the research, formulations will be available for real machining tests. If vegetable oil-in-water mixtures make good metalworking fluids, an important new market may develop for the industrial use of vegetable oils.

2000-01864 Cellulose Diacetate Fibers of Enhanced Biodegradability and Air Filtration

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Grant 00-35504-9395; $155,000; 3 Years

This proposal, though quite fundamental in nature, has a number of practical aspects, which will be of benefit to agriculture, industry and the environment. It deals with the modification of a widely used fiber, namely cellulose diacetate. This fiber is used extensively in filtration devices. The efficiency of a filtration device depends on the amount of surface area available to capture particles, etc. The objective of the proposed research is to significantly increase the surface area of cellulose diacetate fibers which will increase their filtration efficiency. In addition, an increase in surface area will increase the rate of degradation of the fibers, which will result in a decrease in land-fill requirements for discarded filtration devices. The research will be of benefit to agriculture. Cellulose dicacetate is prepared from agriculture products such as wood and cotton. The increase in filtration efficiency will lead to an increased use of cellulose diacetate, which will result in increased demands for the production of wood and cotton.

2000-01827 Film Properties of Chemically Modified Wheat Gluten Binders for Pigment Printing

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Grant 2001-35504-10107; $101,000; 2 Years

The objective of this study is to develop wheat gluten binders for pigment printing of textiles. The development of these binders is important to the textiles printing industry because gluten binders will reduce the formaldehyde that is liberated during the curing of textile print pastes. Melamine formaldehyde auxiliary resins are widely used to enhance the fastness of print pastes to laundering. It has been clearly demonstrated that gluten has potential to be a good print paste binder. At this point, more research is needed to improve the flexibility and fastness to crocking (rubbing). This will be investigated through the study of gluten films. Experiments will be conducted on the effects of a variety of chemical modifications and the addition of plasticizers on film properties. The ability of various types of modified gluten to absorb formaldehyde will be evaluated. Then various types of modified gluten will be added to print paste formulations and performance of the modified gluten will be evaluated. Although the focus is on the potential of gluten in textile printing, the results will be valuable to those who want to expand the application of wheat gluten films, who want to scavenge vapors from phenol formaldehyde resins, and those who want to develop adhesives from wheat gluten. This could provide a large volume end-use for American wheat since 16,000 miles of fabric are printed in the world every day, and each year about 30 million pounds of binders are used in print pastes in the United States.

2000-01828 Process Development and Characterization of Starch Acetate and Biodegradable Synthetic Polymer Blends

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Grant 00-35504-9332; $100,000; 2 Years

Plastics consumption continues to rise, worldwide. Only a small portion of used plastics is recycled. The rest is landfilled or burned. This has serious short and long-term environmental pollution implications. Starch and synthetic "biodegradable" substitutes have been studied and developed, some of which are commercially available. Native starch is abundant and inexpensive, but has shortcomings when considered as a plastic substitute. Modification of starch by chemical means, or blending it with other plastics is required to achieve satisfactory functionality in most applications. Starch acetate is one such modified starch, which has improved functionality compared to native starch.

This project proposes to look at ways of blending starch acetate with three commercially available “biodegradable” plastics (one from starch, the other two from petroleum) to manufacture a new class of composite materials. Foams, sheets and thermo-formed articles will be developed. Physical, mechanical, thermal and morphological properties of the composites will be evaluated. Conditions for and rates of biodegradation of the composites will be studied. The composites are expected to be both functional and biodegradable. Results of this project will enhance the development of new uses for agricultural products and will benefit the environment.

2000-01888 Fundamentals of Water-Only Hydrolysis for Advanced Biomass Pretreatment

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Strengthening Award; Grant 2001-35504-10041; $103,000; 2 Years

Biological processing of abundant lignocellulosic biomass sources such as agricultural residues and ultimately dedicated crops to fuels and chemicals in advanced biorefineries would create major new markets for agriculture and rural manufacturing. Such processes are powerful routes to sustainable production of organic fuels and chemicals on a large scale with unparalleled potential for environmental, economic, and strategic benefits. Enzyme-based technologies offer high product yields vital to achieving these ends, but pretreatment needed to obtain such yields is the typically the most expensive single step with significant impacts on other costs. Recently, technologies based on flow of water through biomass have been shown to promise significant cost reductions but suffer from some important limitations. The overall objective of this proposal is to better understand the mechanisms that differentiate these advanced processes from conventional water-only technologies and build a foundation to improve pretreatment technologies. Because short-chained oligomer intermediates could be a key to explaining such differences, novel models will be developed that focus on the kinetics of oligomer formation, mass transfer, and reaction. Their predictions will be compared to those for currently applied models and data from the literature. New data will then be collected for water-only pretreatment and used to assess the applicability of the various models. The goal is to provide new data, models, insight, and mechanisms that will facilitate the development and commercialization of lower cost advanced pretreatment technologies and speed applications for both new and existing technologies by increasing confidence in their commercialization.

2000-01910 Development, Characterization, and Selection-based Improvement of *S. cerevisiae* Strains Expressing Heterologous Cellulase Enzymes

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Strengthening Award; Grant 00-35504-9495; $130,000; 2 Years

An interdisciplinary research project is proposed with tasks defined by the following:
(1) Develop strains of *Saccharomyces cerevisiae* that express a functional cellulase system allowing anaerobic growth and ethanol production on crystalline cellulose. (2) Characterize cellulose utilization by recombinant strains and test the hypothesis that improved strains can be selected via continuous culture. Haploid laboratory strains will be developed that co-express and secrete functional and complementary cellulases. Expression in industrial yeast strains will also be undertaken. Characterization work associated with objective 2 will allow evaluation of cellulose utilization by first-generation strains. Selection in continuous culture will be investigated as a means to augment molecularly-based approaches for development of superior strains and perhaps enzymes. These objectives advance applied capability and fundamental understanding relative to consolidated bioprocessing (CBP) - a promising strategy for processing cellulosic biomass in which production of cellulase enzymes, cellulose hydrolysis, and

fermentation of resulting soluble sugars occur in a single process step. CBP has marked potential to lower the cost of producing fuels and chemicals from agricultural residues (e.g., corn stover) and crops such as crops such as switchgrass.

2000-01229 Producing Energy From Feedlot Manure Waste Using a Bi-phasic Fermentation System

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Seed Grant; Grant 2001-35501-10100; $75,000; 2 Years

A significant amount of manure waste is generated each year from livestock industry on the state and national level. The manure waste is a potential source of air, surface water and groundwater contamination. The uncontrolled methane release from manure waste is also a concern for global warming. In an effort to develop alternative waste management techniques for manure, the proposed research will evaluate the technical and economical feasibility of producing energy from manure waste by converting the organic waste into methane gas. The methodology is based on a recently developed technology, which has shown promises to produce high quality methane gas from organic waste with desirable economic return.

2000-01797 A Novel Spouted Bed Bioreactor for Solid State Fermentation for Production of Enzyme and Recombinant Protein from Plant Biomass

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Grant 2001-35504-10105; $140,000; 2 Years.

Solid state fermentation (SSF) offers higher production rates and easier product recovery in many cases compared to submerged fermentation (SmF), along with the ability to use many agricultural commodities and byproducts, such as corn and wheat bran, as substrates. By virtue of its use of plant biomass as a substrate, SSF can become a sustainable system of chemical production from natural resources, providing economic benefit to US agriculture and increasing national competitiveness. However, large-scale SSF processes are rare in Western countries. Drawbacks that discourage industrial application include solids handling difficulties, heat and mass transfer limitations, and lack of kinetic and design data for process scale up.

A novel gas-solid spouted bed bioreactor that can overcome problems suffered by the conventional SSF systems have been recently developed in our laboratory. However, more fundamental knowledge and practical demonstrations are needed to determine the feasibility of scaling-up the spouted bed solid state fermentation process. To reach this goal, we will: (1) investigate enzymes (amylases) and heterologous protein (GFP) production in SSF by recombinant filamentous fungi grown in the spouted bed bioreactor, and (2) study hydrodynamics of the spouted bed bioreactor to evaluate its feasibility in scale-up. Useful understanding will be added to the body of knowledge about SSF. The new process may produce industrially important bioactive substances from solid plant biomass at reduced costs. Furthermore, the spouted bed bioreactor also may be used in simultaneous saccharification and fermentation processes for biochemicals production from biomass containing starch or cellulose.

2000-01887 Production of Cellulosic Microfibers

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Grant 00-35504-9357; $170,500; 3 Years.

The primary goal of this project is development of manufactured cellulosic microfibers from wood pulp, as well as from agricultural by-products. Two approaches will be used, both involving lyocell solutions, which are cellulose dissolved in an environmentally benign solvent. The first approach, based on current processes for making manufactured fibers, will employ modifications developed to induce fine microfibers to separate from the solvent. The second approach is based on melt blowing technology for producing nonwoven fabrics. Melt blowing produces very fine fibers, but has only been used as a process with synthetic polymer melts. The results of this study should provide information on the feasibility of producing absorbent microfibers of cellulose, an abundant agricultural resource. Absorbent textile products, particularly nonwovens, have a variety of uses and microfibers increase the surface areas to enhance moisture absorption, as well as filtration properties. The proposed work will combine advanced textile spinning technologies with the new lyocell process for making manufactured cellulosic fibers.

2000-01867 Factors Affecting Solvent Production in *Clostridium acetobutylicum*

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Grant 00-35004-9269; $210,000; 3 Years

The objective of the proposal is to increase our knowledge of the molecular processes which control and limit the production of the solvents acetone and butanol in the anaerobic bacteria *Clostridium acetobutylicum.* This organism has excellent potential for the industrial production of chemicals (e.g., butanol which mixes better with hydrocarbon fuels than ethanol), and the organism is able to grow on a variety of biomass materials. Experiments proposed at this stage are devoted to analysis and modification of the lipid components of the cell membrane. The composition of the membrane is considered a major factor in tolerance to solvents. If the strains can be adapted to be more solvent tolerant, this enhances the prospects for generating higher yielding strains.

We also plan to investigate the genes and enzymes involved in the utilization of xylan, a major component of agricultural biomass. These studies will seek to improve growth and production of solvents by the organism on mixed feedstocks.

2000-01869 Production of Cellulases in Transgenic Alfalfa for Use in Biomass Conversion

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Grant 98-35504-6341; $141,000; 2 Years

This research project is a multidisciplinary feasibility study on producing industrially important enzymes using alfalfa fields as "factories". The overall goals of the research are to develop genetically engineered alfalfa that produces high levels of industrially important enzymes and to develop the technology needed to extract these enzymes from alfalfa juice. Thus, this proposal attempts to use biotechnology to develop new non-food products from existing agricultural resources. The production of value-added crops will aid rural economies and could lead to a reduction in federal commodity support. Technology developed for extraction of these enzymes could be used to extract other value-added products from alfalfa (such as tannins and saponins) and other field grown crops. Furthermore, the availability of large amounts of enzymes will fuel industries such as biopulping, biomass conversion and bioremediation.

Specifically, this research will determine if cellulases can be produced in transgenic alfalfa. The largest single market for cellulases is the production of ethanol from waste lignocellulose. An abundant inexpensive supply of cellulases would have a major impact on the entire biofuels industry. Expression of cellulases in alfalfa may make alfalfa fiber more suitable for use as a feedstock in biomass conversion. Inexpensive supplies of ethanol could replace or reduce the use of gasoline. This would reduce the nation's strategic vulnerability and lower trade deficits. There are also beneficial environmental effects. Automobile emissions are improved by burning ethanol or a mixture of gasoline and alcohol, and producing alcohol from sustainable crops does not contribute to the accumulation of carbon dioxide in the environment.

2000-01164 Bioreactor Production of Secondary Metabolites from Plant Cell Cultures Valluri, J. V.

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Equipment Grant; Grant 2001-35501-10022; $25,000; 1 Year

Bioreactor applications of plant cell cultures allow scientists to isolate unlimited supplies of biologically active chemicals. The plant kingdom continues to be a rich reservoir of pharmaceuticals, from the most common one, aspirin, to the recently discovered anti-cancer drug, Taxol. Large markets also exist for essential oils and other secondary metabolites that are normally obtained by extraction from intact plants. Sandalwood trees are slow growing, endangered tropical plants. Sandalwood is threatened by mycoplasmal infections. Bioreactor grown sandalwood cultures are useful because they do not suffer from diseases, pests and climatic constraints, which limit the use of field grown plants. Our studies on bioreactor culture of sandalwood *(Santalum album* L.) focus on cultural factors and environmental signals that influence essential oil production, making a commercially viable substitute for the sandalwood trees. The mechanisms of environmental signal recognition by plants and the regulation of gene expression are critical frontiers in improvement of biosynthesis of natural products. Another objective is to understand defense gene regulation. We will demonstrate that sandalwood cell suspensions can be developed and successfully cultured in a modified airlift bioreactor. Treatments of sandalwood cells with light, heat and fungal homogenates serve as a model to study the regulation of essential oil production. Investigations will be undertaken on sandalwood to exploit bioreactor technology for clonal propagation of disease-free species and to identify elite cell lines for secondary metabolite production.